Phospholipids of rat tissues after feeding pure phosphatidyl ethanolamine and lecithin

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ABSTRACT Pure phosphatidyl ethanolamine and lecithin from egg yolks were fed to rats in saline or in olive oil and the changes in individual phospholipids in the intestinal wall, liver, and plasma of the animals were studied.

Ingestion of olive oil alone produced increased levels of all phospholipid fractions in each of the three tissues. Feeding phosphatidyl ethanolamine in saline resulted in slightly increased plasma phospholipids, but levels of liver total phospholipids were greatly reduced; when phosphatidyl ethanolamine was fed with olive oil, liver phospholipids were again reduced but this reduction was confined to the phosphatidyl ethanolamine and phosphatidic acid fractions. Feeding lecithin alone did not produce significant changes in levels of plasma or tissue phospholipids.

The results suggest that liver phospholipid synthesis is depressed by feeding phosphatidyl ethanolamine; in the presence of olive oil, hepatic synthesis of phosphatidyl ethanolamine seems to be more selectively inhibited.

KEY WORDS phospholipids · intestinal wall · liver · plasma · force-feeding · olive oil · lecithin · phosphatidyl ethanolamine · inhibition · hepatic synthesis · rats · egg yolk · gradient elution

ISOTOPIC STUDIES on the synthesis of total phospholipids by various animal tissues in vitro have been reported extensively (1, 2) and similar studies in the living animal have been carried out by Artom and Swanson (3, 4). However, less is known of the absorption of purified single phospholipids, with which the present paper is concerned. After we had established that fed phospholipids were absorbed from the intestinal lumen of the rat to the extent of 96% within 4 hr, we studied the effects of feeding purified phosphatidyl ethanolamine (PE) and lecithin on the levels of individual phospholipids of the rat intestinal wall, plasma, and liver after this time interval. Since the phospholipids were fed in saline or in olive oil, the effect of the oil alone on phospholipid synthesis was also studied.

In a previous study, Hill, Linazasoro, Chevallier, and Chaikoff (5) (who were concerned mainly with glucose metabolism in relation to fat feeding) observed that feeding certain fats to rats over a period of 3 days with 50% glucose in the diet did not affect the lipid levels of the liver or the phospholipid levels of plasma. It appears from the present work that feeding fat alone does influence the levels of phospholipids in the liver and the plasma. The changes in levels of plasma and liver phospholipids produced during a single feeding are obviously due to a combination of endogenous changes and absorption from the intestine. The effects of absorption have been further studied by feeding radioactively labeled phospholipids. These results will be reported later.

MATERIALS AND METHODS

Animals

Male Wistar albino rats, 8–12 wk of age and weighing 150–200 g, were used. Before feeding, the animals were randomly distributed amongst the cages and left overnight with water but without food. Materials were administered by stomach intubation using rubber catheters (English gauge 3). The animals were lightly anesthetized with ether so that they were fully recovered by the time the catheter was removed (about 30 sec). The total volume fed was usually 1.5 ml and this was followed by 0.5 ml of saline.

Preparation of Food

Phospholipid (250 mg) or olive oil (400 mg) or 250 mg

Abbreviations: PA, phosphatidic acid; PE, phosphatidyl ethanolamine; LPE, lysophosphatidyl ethanolamine; TLC, thin-layer chromatography.

phospholipid + 400 mg of olive oil were mixed with egg white (0.15 ml) and saline to give a total volume of 1.5 ml. The mixture was emulsified in an ultrasonic vibrator with external cooling (0°C) until it was homogeneous (2–5 min). Sufficient food for 8–10 animals was prepared in the above proportions and 1.5 ml of emulsion was fed from a syringe to each animal as described above. The amount of lipid administered was calculated from the organic phosphorus content of the food in the case of phospholipids and from the total ester content in the case of olive oil, after subtracting the amount of residual lipid in the catheter and feeding vessel.

Preparation of Pure PE and Lecithin from Eggs

Egg yolks were stirred with a little water and a volume (x ml) of the mixture poured into a mechanically stirred mixture of chloroform ($3x$ ml) and ethanol ($6x$ ml). The mixture was heated under reflux (in an atmosphere of nitrogen) for 15 min. The precipitated proteins were filtered under suction through Whatman No. 1 paper and the filtrate was evaporated under reduced pressure to a low volume. Acetone (20 vol) was added and the mixture allowed to stand at 4°C overnight. The precipitated crude phospholipids were collected by filtration. They were redissolved in $CHCl_3$–CH_3OH 1:1 (v/v) 50 ml, and the extract was washed with 0.1 N HCl (3 vol). After standing for some hours the $CHCl_3$ layer was separated and the solvent was evaporated in vacuo.

The residual crude phospholipids (which still contained a substantial amount of triglycerides) were dissolved in dry chloroform and further purified by chromatography.

Silicic acid (Mallinckrodt Chemical Works, New York) 100 mesh was treated as described by Billimoria, Curtis, and Maclagan (6). A portion of the dry $CHCl_3$ extract containing phospholipid (not exceeding 5 g) was applied to a column (5.6 cm diam) containing 150 g of silicic acid and the column was eluted with dry $CHCl_3$ (3 liters) to remove all triglycerides and free and esterified cholesterol. The column was transferred to a gradient elution apparatus (7) and the individual phospholipids were separated by elution with a linearly increasing concentration of CH_3OH in $CHCl_3$. The separation was monitored by phosphorus estimations and the separated peaks of PE, lysophosphatidyl ethanolamine (LPE), lecithin, and sphingomyelin were individually pooled. PE and lecithin fractions were analyzed as described under chemical estimations.

Rat Tissue Phospholipids

Fed animals were anesthetized with ether 4 hr after feeding. Blood was collected from the heart and after exsanguination the animals were killed. The liver, intestine, and stomach were removed and the intestinal walls were slit and washed thoroughly with saline. Stomach washings were similarly obtained. Phospholipids and esters in the washings were estimated after extraction with Bloor's solvent (ethanol–ether 3:1) in order to determine the amount of lipid not absorbed.

The intestine and liver were separately ground with pestle and mortar under Bloor's solvent and then thoroughly extracted with several further portions of the boiling solvent. The extracts were filtered and made up to a known volume. An aliquot was removed for estimation of organic phosphorus. The solvent was removed from the residual extract and the crude lipids were dissolved in dry $CHCl_3$, filtered under centrifugal force through a Seitz filter, and chromatographed.

Blood was centrifuged for 10 min at 4°C at 3,500 × g and the plasma separated. The latter (1 vol) was extracted with Bloor's solvent and made up to 20 vol with the solvent. An aliquot was reserved for phosphorus estimation and the rest of the extract was chromatographed.

Usually phospholipids of liver or intestine from the organs of individual animals were fractionated, but plasma (8–10 ml) was pooled from at least two animals in order to obtain sufficient material.

Identification of Phospholipids

The purified phospholipid fractions were identified by TLC. Organic phosphorus was estimated by a modification of the method of Allen (8), as described previously (6). Values of phospholipids were obtained by multiplying the phosphorus content by 25. Amino nitrogen was estimated by the method of Lea and Rhodes (9), except that the ninhydrin reagent was prepared according to Jacobs (10). Total nitrogen was estimated after digestion of the lipid with perchloric acid (11), using the Nessler reagent according to King and Wootton (12); NaI was substituted for KI in the Nessler reagent to avoid formation of the insoluble potassium perchlorate. Choline was determined according to Böttcher, Pries, and Van Gent (13). Bases were identified by amino acid chromatography after hydrolysis of the lipids with 6 N HCl at 100°C in sealed tubes for 6 hr (14). The inositol content of each fraction was determined by the microbiological assay of Norris and Darbre (15) and glycerophosphoryl bases were identified by the paper chromatographic technique of Dawson (16). Ester estimations were carried out according to Rapport and Alonzo (17). The fatty acid compositions of the lipids fed were determined by gas–liquid chromatography on ethylene glycol adipate polyester (15% on Celite, 100–120 mesh) at 174°C and a flow rate of 44 ml/min using an argon ionization detector as previously described by Billimoria, Irani, and Maclagan (18).

RESULTS

Purity of Lipids Fed

The PE fraction gave a single spot on TLC and behaved identically with a sample of synthetic PE prepared by Billimoria and Lewis (19). Its nitrogen was all present as amino nitrogen and the P/N ratio was 1:1.01. A trace of serine was detected by paper chromatography after hydrolysis. The ester/P ratio (1.91:1.0) was a little lower than the theoretical value of 2.0:1. Some (1–3%) of the PE was present in the plasmalogen form. LPE was completely absent as indicated by a negative red cell lysis test and by TLC.

Lecithin was free from sphingomyelin and gave a P/N ratio of 1:1.01, showed complete absence of amino nitrogen, and gave a P/ester ratio of 1:2.0.

TABLE 1 FATTY ACID COMPOSITION OF EGG YOLK PHOSPHOLIPIDS

	PE	Lecithin
	% of total fatty acid	
Myristic	tr.	tr.
Palmitic	17.8	43.0
Palmitoleic	—	tr.
Stearic	33.4	9.5
Oleic	25.7	27.0
Linoleic	9.5	16.0
Arachidonic	10.7	1.7
Behenic	2.9	3.0

Tissue Phospholipid Fractions

The fraction shown in Fig. 1 as phosphatidic acid contained only a trace of nitrogen as impurity and on TLC ran close to the solvent front, its R_f being identical with a synthetic marker of β,γ-dipalmitoyl L-α-phosphatidic acid prepared by the method of Hessel, Morton, Todd, and Verkade (20). After mild alkali hydrolysis, chromatography (16) established the presence of glycerophosphoric acid. The PE fractions from plasma, liver, and intestine were pure but the lecithin fractions contained some sphingomyelin. Further separation of the lecithin from sphingomyelin was unnecessary as lecithin and sphingomyelin phosphorus could be separately estimated from the mixture after mild alkali hydrolysis by the method of Dawson (21). The LPE fraction, although giving on TLC only one spot corresponding to the required lipid, contained traces of adhering amino acids in spite of the washing procedure described in the Methods

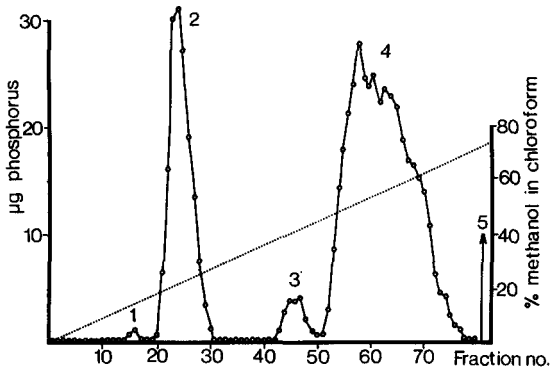

FIG. 1. Separation of egg yolk phospholipids by continuous linear gradient elution chromatography. Crude phospholipids (4.14 g) were applied to silicic acid (150 g). Column was eluted with $CHCl_3$–CH_3OH with increasing concentration of CH_3OH (right-hand ordinate). Fractions of 25 ml were collected. Micrograms of P from an aliquot (0.1 ml) are plotted against fraction number. Broken line, gradient of CH_3OH–$CHCl_3$. Peaks: *1*, phosphatidic acids; *2*, PE; *3*, LPE containing 20% w/w phosphatidyl inositol; *4*, lecithins; *5*, sphingomyelins and lysolecithins (250 mg), eluted with C_2H_5OH–$CHCl_3$–H_2O 6:3:1 (v/v).

TABLE 2 INTESTINAL WALL PHOSPHOLIPIDS 4 HR AFTER FEEDING LIPIDS
Results expressed as mg/100 g tissue ± SEM with statistical significance of difference from control values in parentheses

	Lipids Fed					
	None	Olive Oil	PE/Saline	Lecithin/Saline	PE/Olive Oil	Lecithin/Olive Oil
No. of expts. (animals)	6 (6)	6 (6)	7 (7)	8 (8)	8 (8)	6 (6)
PA	19.5 ± 5.7	9.8 ± 1.2 (NS)	31.6 ± 3.3 (NS)	25.1 ± 2.1 (NS)	58.8 ± 10.0 ($P < 0.01$)	28.8 ± 3.5 (NS)
PE	159.3 ± 26.2	210.8 ± 18.7 (NS)	109.4 ± 10.2 (NS)	170.1 ± 11.5 (NS)	113.5 ± 10.8 (NS)	155.0 ± 9.3 (NS)
LPE	45.1 ± 1.6	58.6 ± 4.9 ($P < 0.05$)	44.4 ± 4.8 (NS)	41.5 ± 4.2 (NS)	59.0 ± 3.1 ($P < 0.01$)	46.4 ± 3.8 (NS)
Total cephalin (PE + LPE)	204.4 ± 28.6	269.4 ± 21.5 (NS)	153.8 ± 8.3 (NS)	211.6 ± 11.5 (NS)	172.5 ± 11.6 (NS)	201.4 ± 9.3 (NS)
Lecithin	281.0 ± 43.7	392.8 ± 18.6 ($P < 0.05$)	205.5 ± 19.1 (NS)	374.3 ± 42.6 (NS)	251.3 ± 20.3 (NS)	326.2 ± 19.4 (NS)
Sphingomyelin	44.4 ± 3.5	63.3 ± 2.3 ($P < 0.001$)	43.5 ± 3.1 (NS)	64.2 ± 9.6 (NS)	45.4 ± 3.3 (NS)	61.4 ± 5.1 ($P < 0.05$)
Total phospholipids	549.3 ± 80.6	735.2 ± 39.9 ($P = 0.05$)	434.3 ± 24.3 (NS)	675.2 ± 47.2 (NS)	528.0 ± 31.9 (NS)	617.9 ± 33.2 (NS)

TABLE 3 PLASMA PHOSPHOLIPIDS 4 HR AFTER FEEDING LIPIDS
Results expressed as mg/100 ml plasma ± SEM with statistical significance in parentheses

	Lipids Fed					
	None	Olive Oil	PE/Saline	Lecithin/Saline	PE/Olive Oil	Lecithin/Olive Oil
No. of expts. (animals)	4 (53)	4 (48)	4 (8)	4 (8)	4 (8)	4 (8)
PA	1.0 ± 0.3	1.2 ± 0.1 (NS)	5.0 ± 1.7 (NS)	0.9 ± 0.1 (NS)	4.4 ± 1.2 ($P < 0.05$)	1.3 ± 0.2 (NS)
PE	4.6 ± 1.4	8.1 ± 1.2 (NS)	11.7 ± 3.5 (NS)	2.8 ± 0.8 (NS)	11.4 ± 0.8 ($P < 0.01$)	3.6 ± 0.6 (NS)
LPE	2.9 ± 0.9	7.1 ± 1.1 ($P < 0.05$)	7.9 ± 2.7 (NS)	4.0 ± 0.9 (NS)	3.5 ± 1.1 (NS)	2.3 ± 0.5 (NS)
Total cephalin (PE + LPE)	7.5 ± 0.5	15.1 ± 2.3 ($P < 0.02$)	19.0 ± 3.2 ($P < 0.01$)	6.8 ± 1.5 (NS)	14.9 ± 1.0 ($P < 0.001$)	5.9 ± 0.8 (NS)
Lecithin	83.2 ± 3.1	110.1 ± 3.7 ($P < 0.01$)	120.0 ± 13.9 (NS)	82.6 ± 7.3 (NS)	106.7 ± 5.7 ($P < 0.02$)	98.5 ± 1.3 ($P < 0.01$)
Sphingomyelin	7.9 ± 0.7	11.7 ± 0.2 ($P < 0.01$)	11.4 ± 1.3 ($P < 0.05$)	9.1 ± 0.2 (NS)	12.1 ± 1.7 (NS)	9.2 ± 1.2 (NS)
Total phospholipids	99.6 ± 1.9	138.1 ± 3.5 ($P < 0.001$)	137.9 ± 15.5 ($P < 0.05$)	99.4 ± 5.3 (NS)	137.9 ± 6.9 ($P < 0.01$)	115.0 ± 2.6 ($P < 0.01$)

TABLE 4 LIVER PHOSPHOLIPIDS 4 HR AFTER FEEDING LIPIDS
Results expressed as mg/100 g tissue ± SEM with statistical significance in parentheses

	Lipids Fed					
	None	Olive Oil	PE/Saline	Lecithin/Saline	PE/Olive Oil	Lecithin/Olive Oil
No. of expts. (animals)	6 (12)	6 (12)	9 (9)	8 (8)	8 (8)	8 (8)
PA	147.4 ± 40.8	284.3 ± 23.7 ($P < 0.02$)	62.9 ± 12.8 (NS)	137.1 ± 18.4 (NS)	89.1 ± 11.0 (NS)	123.4 ± 11.7 (NS)
PE	934.8 ± 103.2	1225.8 ± 19.4 ($P < 0.02$)	595.9 ± 28.8 ($P < 0.01$)	942.9 ± 64.8 (NS)	469.5 ± 71.7 ($P < 0.01$)	920.0 ± 30.6 (NS)
LPE	77.0 ± 9.9	59.0 ± 2.8 (NS)	61.7 ± 5.9 (NS)	120.3 ± 10.8 ($P < 0.02$)	97.6 ± 11.4 (NS)	110.9 ± 12.1 ($P < 0.05$)
Total cephalin (PE + LPE)	1011.8 ± 99.1	1284.7 ± 21.3 ($P < 0.05$)	657.6 ± 33.3 ($P < 0.01$)	1063.2 ± 70.3 (NS)	567.1 ± 81.6 ($P < 0.01$)	1030.9 ± 107.8 (NS)
Lecithin	1403.0 ± 81.5	1814.9 ± 45.8 ($P < 0.001$)	1076.6 ± 111.5 ($P < 0.05$)	1610.3 ± 77.9 (NS)	1398.9 ± 51.9 (NS)	1628.4 ± 165.1 (NS)
Sphingomyelin	93.0 ± 5.5	124.6 ± 5.1 ($P < 0.01$)	105.8 ± 14.1 (NS)	119.3 ± 10.2 ($P < 0.05$)	79.1 ± 11.4 (NS)	124.9 ± 14.5 (NS)
Total phospholipids	2655.3 ± 129.0	3508.5 ± 38.2 ($P < 0.001$)	1901.2 ± 85.1 ($P < 0.001$)	2930.1 ± 151.6 (NS)	2134.1 ± 86.9 ($P < 0.01$)	2907.6 ± 260.4 (NS)

section. This fraction also contained almost all the inositol. Over 80% of this fraction was present as LPE, the remaining 20% being phosphatidyl inositol. The red cell lysis reaction was strongly positive.

A separation of the egg phospholipids on silicic acid columns by linear gradient elution is shown in Fig. 1. Only the PE and lecithin peaks were used in the feeding experiments.

The fatty acid compositions of the PE and lecithin fed are shown in Table 1. Of the saturated acids PE contained mainly stearic acid whereas lecithin contained mainly palmitic acid. Of the unsaturated acids the oleic acid content was similar in both lipids but PE contained more arachidonic acid and lecithin more linoleic acid.

Levels of phospholipid fractions in the intestinal wall, plasma, and liver after feeding olive oil and (or) phospholipids are shown in Tables 2, 3, and 4 respectively. In the plasma of unfed animals the major phospholipid is lecithin (83%) and only 8% of cephalin is present. In the liver and intestine higher levels of cephalin (38%) are found. Relatively low levels of LPE (3%) are found in plasma and liver but as much as 8% is present in intestine. The reported phospholipase A activity (22) in intestinal mucosa may account for the higher LPE content of the organ. It is unlikely that this is a cleavage product of either PE or plasmalogen arising as an artifact of chromatography since, under similar conditions, synthetic PE and the small amounts of plasmalogen PE from egg yolk are recovered intact.

The effects of feeding PE differed significantly from those of feeding lecithin, and these differences are summarized in Table 5. The major phospholipid fractions of

TABLE 5 DIFFERENCES IN TISSUE PHOSPHOLIPIDS AFTER FEEDING PE AND LECITHIN

Results given as the difference (amount when lecithin fed) − (amount when PE fed) ± SEM. The statistical significance is shown in parentheses

	Intestine	Plasma	Liver
	mg/100 g wet wt	*mg/100 ml*	*mg/100 g wet wt*
PA	−10.1 ± 3.9 ($P < 0.05$)	−4.1 ± 1.7 ($P = 0.05$)	+74.2 ± 22.4 ($P < 0.01$)
PE	+60.7 ± 15.3 ($P < 0.01$)	−8.9 ± 3.6 ($P = 0.05$)	+347.0 ± 70.9 ($P < 0.001$)
LPE	−2.9 ± 6.3 (NS)	−3.9 ± 2.8 (NS)	+58.6 ± 12.2 ($P < 0.001$)
Total cephalin	+57.8 ± 14.1 ($P < 0.01$)	−12.8 ± 3.5 ($P < 0.02$)	+405.6 ± 77.7 ($P < 0.001$)
Lecithin	+168.8 ± 46.7 ($P < 0.01$)	−19.4 ± 15.7 (NS)	+533.7 ± 136.0 ($P < 0.01$)
Sphingomyelin	+20.7 ± 10.1 (NS)	−2.3 ± 1.3 (NS)	+13.5 ± 17.4 (NS)
Total phospholipids	+240.9 ± 53.1 ($P < 0.001$)	−40.8 ± 16.4 ($P = 0.05$)	+1028.9 ± 174.1 ($P < 0.001$)

liver and intestine were increased and those of plasma decreased when PE was replaced by lecithin in the feeding experiments.

DISCUSSION

Our results indicate that a single meal of olive oil gives rise to large changes in phospholipid levels of plasma, intestinal wall, and the liver of the rat, which were raised by as much as 30–40% in each of the tissues studied. These increases were particularly prominent in the liver and were distributed over almost all the phospholipid fractions.

In fat-feeding experiments Hill et al. (5) have shown that the feeding of fat-containing diets (corn oil and vegetable oils) over a period of 3 days did not influence the lipid content of the liver or the lipid, including phospholipid, levels of the plasma. The striking difference in our results may be due to differences in dietary composition. Whereas the diet of Hill et al. contained as much as 50% glucose we fed fat alone. The difference in the two results is of sufficient interest to warrant further investigation as it is conceivable that administration of fat alone may result in a fatty liver, which may be avoided by the addition of carbohydrate to the feed.

The two pure lipids, PE and lecithin, have totally different effects on lipid levels of plasma, intestine, and liver when fed individually.

On feeding PE the levels of total phospholipids in the liver were strikingly reduced; this reduction was observed both in the lecithin and PE fractions. A similar trend was observed in the intestine, although the changes here were not significant. The larger scatter of the results is probably due to absorption from the intestine still occurring 4 hr after feeding. The plasma total phospholipids were raised after the feeding but the plasma PE level was not significantly increased, the increase being uniformly distributed over the other phospholipid fractions.

Two possible explanations of the reduced content of liver phospholipids after feeding PE are (*a*) inhibition of liver synthesis of phospholipids or (*b*) increase in the rate of destruction of phospholipids. The latter suggestion seems less likely, as an increased destruction would be associated with increased levels of the intermediate metabolite PA which were not observed; in fact, PA levels were reduced from 147 to 63 mg/100 g wet weight, suggesting decreased breakdown. When PE was fed with olive oil, the liver PE was decreased by over 60%, whereas the lecithin levels were almost unchanged. It is therefore suggested that the reduced levels of phospholipids represent an inhibition of synthesis. The subject is still under investigation.

Similar results were not observed when lecithin was fed; instead the lecithin contents of intestine, plasma, and liver were all slightly raised. The fact that plasma and liver lecithin levels are not significantly raised after feeding lecithin suggests that its turnover must be very rapid, in agreement with Gurr, Pover, Hawthorne, and Frazer (23).

Our interest in the experiments was concerned partly with the well known effects of fat feeding on blood coagulation, which appear to depend mainly on the PE fraction (24). Our results indicate that feeding PE and (or) olive oil causes large increases in the plasma PE fraction, which would explain the accelerated coagulation. Feeding lecithin with olive oil, however, abolishes the rise in plasma PE; lecithin might therefore merit trial as a desirable adjuvant to dietary fats.

We are grateful to Mr. J. E. Bozzino for skilled technical assistance.

The expenses of the work were defrayed by grants from the

Medical Research Council and the Endowment Funds of the Westminster Hospital.

Manuscript received 8 September 1965; accepted 15 November 1965.

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